

=> file hca;d que 19

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FILE COVERS 1967 - 26 Aug 1995 (950826/ED) VOL 123 ISS 9

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```
L1      2639 SEA FILE=HCA (ALPHA(1A) INTERFERON) /IT
L2      29 SEA FILE=HCA HAUPTMANN RUDOLF/AU
L3      6 SEA FILE=HCA L1 AND L2
L4      29763 SEA FILE=HCA INTERFERON#/IA, IT, ST
L5      33396 SEA FILE=HCA PLASMID AND EPISOME/IT
L6      61528 SEA FILE=HCA L5 OR (PLASMID# OR EPISOME#)/IA, IT, ST
L7      984 SEA FILE=HCA L4 AND L6
L8      3 SEA FILE=HCA L7 AND (142192-09-4 OR 142192-09-4D OR 14219
          2-09-4P)
L9      2 SEA FILE=HCA L8 NOT L3
```

=> d bib abs hitrn 1-

```
L9      ANSWER 1 OF 2 HCA COPYRIGHT 1995 ACS
AN      117:169442 HCA
TI      Manufacture of O-glycosylated human interferon .alpha.
IN      Adolf, Guenther; Himmler, Adolf; Ahorn, Horst Johann; Kalsner, Inge;
        Maurer-Fogy, Ingrid
PA      Boehringer Ingelheim International G.m.b.H., Germany
SO      PCT Int. Appl., 98 pp.
        CODEN: PIXXD2
PI      WO9201055 A1 920123
DS      W: AU, CA, CS, FI, HU, JP, KR, NO, PL, SU, US
        RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE
AI      91WO-EP01266 910706
PRAI    90DE-4021917 900710
        90DE-4035877 901112
DT      Patent
LA      German
AB      A human interferon .alpha. is manufd. in a glycosidated form by expression of the corresponding cDNA in animal cell culture. Expression vectors for animal cells using an SV40 replication origin, a cytomegalovirus promoter, and a dihydrofolate reductase minigene were prep'd. and interferon .alpha.2c cDNA was introduced into them. CHO cells were transformed with these plasmids and transformants challenged with methotrexate to amplify the plasmid. Lines resistant to 5000 nM methotrexate yielded 190,000-960,000 interferon units/mL medium. The purified protein was glycosidated and showed the expected N- and C-terminal peptides. Glycosidation sites were identified.
IT      142192-09-4, Interferon .alpha.2 (human clone pAD19B-IFN protein moiety reduced)
        (amino acid sequence of, complete, and expression in CHO cells of cDNA for)
```

L9 ANSWER 2 OF 2 HCA COPYRIGHT 1995 ACS
AN 117:46559 HCA
TI Glycosidated **interferon** .alpha. manufacture with
transgenic animal cells
IN Himmler, Adolf; Adolf, Guenther
PA Boehringer Ingelheim International G.m.b.H., Germany
SO Ger. Offen., 24 pp.
CODEN: GWXXBX
PI DE4021917 A1 920116
AI 90DE-4021917 900710
DT Patent
LA German
AB An expression vector for the manuf. of human **interferon**
.alpha., specifically .alpha.2 or .alpha.2C, in animal cell culture
to ensure normal glycosidation of the protein are described. The
plasmid uses a cytomegalovirus enhancer and promoter coupled
to a hybrid intron (cytomegalovirus donor region, Hb acceptor
region) to drive expression of the cDNA. Construction of the
expression vector by std. methods is described. General purpose
expression vectors derived from this expression vector are also
described.
IT 142192-09-4DP, **Interferon** .alpha.2 (human clone
pAD19B-IFN protein moiety reduced), O-glycosidated
142192-09-4P, **Interferon** .alpha.2 (human clone
pAD19B-IFN protein moiety reduced)
(manuf. in animal cell culture of)

Exmr: C. Smith (AU 1812)

=> file hca;d que 120;d iall 1-
FILE 'HCA' ENTERED AT 14:58:20 ON 30 AUG 95
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L1	2639 SEA FILE=HCA (ALPHA(1A)INTERFERON) /IT
L2	29 SEA FILE=HCA HAUPTMANN RUDOLF/AU
L3	6 SEA FILE=HCA L1 AND L2
L4	29763 SEA FILE=HCA INTERFERON#/IA,IT,ST
L10	131526 SEA FILE=HCA (ESCHERICHIA COLI OR E COLI)/IA,IT,ST
L11	1366 SEA FILE=HCA L4 AND L10
L12	53776 SEA FILE=HCA (TOXIN#)/IA,IT,ST
L13	87 SEA FILE=HCA L11 AND L12
L17	884 SEA FILE=HCA (HEAT(2A)STABLE(2A)TOXIN# OR STII OR ST11 OR STABLE(2A)ENTEROTOXIN#)/IA,IT,ST
L18	3 SEA FILE=HCA L13 AND L17
L19	2 SEA FILE=HCA L18 NOT L3
L20	2 SOR L19 PY

L20	ANSWER 1 OF 2 HCA COPYRIGHT 1995 ACS
ACCESSION NUMBER:	98:15278 HCA
TITLE:	Cyclic GMP as the second messenger in helper cell requirement for .gamma.-interferon production
AUTHOR(S):	Johnson, Howard M.; Archer, Douglas L.; Torres, Barbara A.
CORPORATE SOURCE:	Dep. Microbiol., Univ. Texas, Galveston, TX, 77550, USA
SOURCE:	J. Immunol. (1982), 129(6), 2570-2 CODEN: JOIMA3; ISSN: 0022-1767
DOCUMENT TYPE:	Journal
LANGUAGE:	English
CLASSIFICATION:	15-5 (Immunoochemistry)
ABSTRACT:	Cyclic GMP and activators (acetylcholine, Escherichia ***coli*** heat-stable toxin) of guanylate cyclase were capable of completely replacing the helper cell or interleukin 2 requirement for .gamma.-interferon (IFN.gamma.) prodn. by Lyt-1-, 2+ cells from C57BL/6 mouse spleen cells. The cyclic GMP help was independent of DNA synthesis or proliferation in the IFN.gamma.-producing cells, because cyclic GMP reversed mitomycin C blockage of IFN.gamma. prodn. but did not reverse the inhibition of DNA synthesis. Thus, the findings presented here are unrelated to the question of the 2nd messenger role of cyclic GMP in the activation of lymphocytes for DNA synthesis and cellular proliferation. The cyclic GMP help for IFN.gamma. prodn. was antagonized by cyclic AMP and inducers (isoproterenol) of adenylate cyclase.

SUPPL. TERM: **interferon** cGMP messenger helper cell
 INDEX TERM: Spleen, metabolism
 (helper cell function of, in .gamma.-
 interferon formation, cyclic GMP and
 guanylate cyclase activities in relation to)
 INDEX TERM: **Interferons**
 (.gamma.-, formation of, cyclic GMP and guanylate
 cyclase replacement of helper cell function in)
 INDEX TERM: 7665-99-8 9054-75-5
 (.gamma.-**interferon** formation requirement
 for helper cell activity replacement by)

L20 ANSWER 2 OF 2 HCA COPYRIGHT 1995 ACS
 ACCESSION NUMBER: 105:41056 HCA
 TITLE: Tumor necrosis factor, compositions containing
 it, DNA encoding it and assay method using this
 DNA
 INVENTOR(S): Aggarwal, Bharat Bhushan; Lee, Sang He; Goeddel,
 David Vannorman; Nedwin, Glenn Evan
 PATENT ASSIGNEE(S): Genentech, Inc., USA
 SOURCE: Eur. Pat. Appl., 90 pp.
 CODEN: EPXXDW

	NUMBER	DATE
PATENT INFORMATION:	EP-168214 A2	860115
DESIGNATED STATES:	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE	
APPLICATION INFORMATION:	85EP-0304758	850703
PRIORITY APPLN. INFO.:	84US-0628059	840705
	84US-0627959	840705
	84US-0628060	840705
	84US-0677454	841203
	84US-0677156	841203
	84US-0677257	841203
DOCUMENT TYPE:	Patent	
LANGUAGE:	English	
INT. PATENT CLASSIF.:		
MAIN:	C07K-003/18	
SECONDARY:	C07K-013/00; A61K-037/02; C12P-021/02; C12N-015/00; C12Q-001/68; A61K-045/02	
INDEX:	A61K-045/02, A61K-037/02	
CLASSIFICATION:	15-1 (Immunochemistry)	
	Section cross-reference(s): 1, 3, 16	

ABSTRACT:
 A method for the isolation and purifn. of tumor necrosis factor (TNF) from recombinant and nonrecombinant cells is presented. Thus, human peripheral blood monocytes were induced with Staphylococcal interotoxin 3 and PMA (a tumor promoter) to produce TNF. The cell culture supernatant contained both TNF and lymphotoxin. To remove the lymphotoxin the TNF activity was batch-absorbed to controlled pore glass beads and eluted, after washing, with 20% ethylene glycol. This eluate was directly applied to a DEAE cellulose 53 column and eluted, after washing, with step up gradients of 75 mM, 150 mM, and 500 mM NaCl in 10 mM phosphate buffer. The eluate was monitored for absorbance at 280 nm and TNF activity as a function of elution fractions. The TNF active fraction was concd., dialyzed, and loaded onto a quaternary ammonium group-substituted

Sepharose bead column and the eluted, after washing, with a linear gradient of 40-75 nM NaCl in an appropriate buffer. The effluent was collected in 2 mL aliquots and monitored for absorbance at 280 nm, cond., and TNF activity. The TNF active fraction was then subjected to chromatofocusing using a Pharmacia Mono P column. One mL aliquots were collected and the absorbance at 280 nm and the pH of the effluent were measured. TNF had an isoelec. point of .apprx.5.3. The mol. wt. of TNF, as detd. by HPLC was .apprx.45,000 daltons. Plasmid vectors that contained TNF- and TNF mutant-coding sequences and that were capable of expressing those sequences in **Escherichia coli**, yeast, and mammalian cells were constructed and the nucleotide and amino acid sequences of the wild-type and mutant TNFs were presented. TNF can be used in the therapeutic treatment of malignant tumors, either alone or in synergistic combination with an interferon.

SUPPL. TERM: human tumor necrosis factor isolation purifn; cloning tumor necrosis factor mutant cDNA; neoplasm inhibitor tumor necrosis factor

INDEX TERM: **Escherichia coli**
Yeast
(cloning in, of tumor necrosis factor cDNA, of human)

INDEX TERM: Protein sequences
(of human tumor necrosis factor and mutants, of human, complete)

INDEX TERM: Molecular cloning
(of tumor necrosis factor cDNA, of human, in **Escherichia coli** and yeast and mammalian cells)

INDEX TERM: Alkenes, polymers
(polymers, in tumor necrosis factor isolation and purifn.)

INDEX TERM: Cytotoxic agents
Neoplasm inhibitors
(tumor necrosis factor and mutants as, genetically engineered)

INDEX TERM: Animal cell
(CHO, cloning in, of tumor necrosis factor cDNA, of human)

INDEX TERM: Glass, oxide
(beads, in tumor necrosis factor isolation and purifn.)

INDEX TERM: Mutation
(deletion, in tumor necrosis factor of human, construction of plasmids encoding)

INDEX TERM: Toxins
(entero-, STII, leader sequence of, of **Escherichia coli**, tumor necrosis factor fusion product with)

INDEX TERM: Mutation
(insertion, in tumor necrosis factor of human, construction of plasmids encoding)

INDEX TERM: Lymphokines and Cytokines
(lymphotoxins, tumor necrosis factor free of, prepn. of)

INDEX TERM: Gene and Genetic element, microbial
(promoter, of alc. dehydrogenase gene, tumor

necrosis factor expression in yeast under
regulation of)

INDEX TERM:

Mutation

(substitution, in tumor necrosis factor of human,
construction of plasmids encoding)

INDEX TERM:

Lymphokines and Cytokines

(tumor necrosis factor, of human, isolation and
purifn. of, from recombinant and nonrecombinant
sources)

INDEX TERM:

Deoxyribonucleic acid sequences

(tumor necrosis factor-specifying, of human)

INDEX TERM:

Interferons

(.gamma.-, tumor necrosis factor administration
with, as neoplasm inhibitor)

INDEX TERM:

94948-61-5	103107-16-0	103107-17-1	103107-18-2
103107-19-3	103107-20-6	103107-21-7	103107-22-8
103107-23-9	103107-24-0	103107-25-1	103107-26-2
103107-27-3	103107-28-4	103107-29-5	103107-30-8
103107-31-9	103107-32-0	103107-33-1	103107-34-2
103107-35-3	103107-36-4	103107-37-5	103107-38-6
103107-39-7	103107-40-0	103107-41-1	103107-42-2
103107-43-3	103107-44-4	103107-45-5	103107-46-6
103107-47-7	103107-48-8	103107-49-9	103107-50-2
103107-51-3	103107-52-4	103107-53-5	103107-54-6
103107-55-7	103107-56-8	103107-57-9	103107-58-0
103107-59-1	103107-60-4	103107-61-5	103107-62-6
103107-63-7	103107-64-8	103107-65-9	103107-66-0
103107-67-1	103107-68-2	103107-69-3	103107-70-6
103107-71-7	103107-72-8	103107-73-9	103107-74-0
103107-75-1	103107-76-2	103107-77-3	103107-78-4
103107-79-5	103107-80-8	103107-81-9	103107-82-0
103107-83-1	103107-84-2	103107-85-3	103107-86-4
103107-87-5	103107-88-6	103255-42-1	

(amino acid sequence of)

INDEX TERM:

9003-53-6

(beads, in tumor necrosis factor isolation and
purifn.)

INDEX TERM:

107-21-1, biological studies 9003-53-6D, quaternary
amino-substituted 9012-36-6D, quaternary ammonium
group-substituted 9013-34-7 12627-13-3

(in tumor necrosis factor isolation and purifn.)

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L1	2639 SEA FILE=HCA (ALPHA(1A)INTERFERON) /IT
L2	29 SEA FILE=HCA HAUPTMANN RUDOLF/AU
L3	6 SEA FILE=HCA L1 AND L2
L10	131526 SEA FILE=HCA (ESCHERICHIA COLI OR E COLI)/IA, IT, ST
L21	4930 SEA FILE=HCA (ALPHA(1A)INTERFERON) /IA, IT, ST
L22	4930 SEA FILE=HCA (142192-09-4 OR 142192-09-4D OR 142192-09-4P)/IA, IT, ST OR L21
L23	360 SEA FILE=HCA L22 AND L10
L24	354 SEA FILE=HCA L23 NOT L3
L26	496 SEA FILE=HCA (PHOA OR ALKALINE(2A) PHOSPHATASE (2A) PROMOTER #) /IA, IT, ST
L27	1 SEA FILE=HCA L24 AND L26

L27 ANSWER 1 OF 1 HCA COPYRIGHT 1995 ACS
ACCESSION NUMBER: 103:17753 HCA
TITLE: Secretion of human interferon-.
alpha. induced by using secretion
vectors containing a promoter and signal
sequence of alkaline phosphatase gene of
Escherichia coli
AUTHOR(S): Miyake, Tetsuo; Oka, Takanori; Nishizawa,
Tsutomu; Misoka, Fusakazu; Fuwa, Toru; Yoda,
Koji; Yamasaki, Makari; Tamura, Gakuzo
CORPORATE SOURCE: Cent. Res. Lab., Wakunaga Pharm. Co., Ltd.,
Hiroshima, 729-64, Japan
SOURCE: J. Biochem. (Tokyo) (1985), 97(5), 129-36
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 3-4 (Biochemical Genetics)
Section cross-reference(s): 13

ABSTRACT:
A new vector was constructed which contains the promoter and the signal sequence of the *E. coli phoA* gene, the structural gene for the periplasmic alk. phosphatase [9001-78-9]. One of the most useful characteristics of this vector is the unique HindIII restriction site located just at the end of the *phoA* signal sequence. This restriction site was generated by oligonucleotide-directed site-specific mutagenesis without changing the amino acid sequence of the signal peptide. Any kind of foreign structural gene can be easily inserted into the HindIII site by using synthetic oligonucleotides to construct a hybrid gene which has neither an extra

02/08/31
10/10/31

sequence nor a deletion between the **phoA** signal sequence and the foreign structural gene. Human **.alpha.-interferon** gene was inserted into this HindIII site. When this hybrid gene was expressed under the control of the **phoA** promoter region, a low but significant activity was recovered in the cold water wash of the cells after an osmotic shock procedure.

SUPPL. TERM: **interferon alpha** gene cloning
 Escherichia; alk phosphatase interferon gene cloning vector; human interferon gene cloning Escherichia

INDEX TERM: **Escherichia coli**
 (cloning in, of **.alpha.-interferon** gene of human)

INDEX TERM: Gene and Genetic element, animal
 (for **.alpha.-interferon**, of human, cloning in **Escherichia coli** of)

INDEX TERM: Molecular cloning
 (of **.alpha.-interferon** gene, of human, in **Escherichia coli**)

INDEX TERM: Gene and Genetic element, microbial
 (promoter, for alk. phosphatase, of **Escherichia coli**, in human **.alpha.-interferon** gene expression)

INDEX TERM: Biological transport
 (secretion, of **.alpha.-interferon**, of human, from **Escherichia coli**, alk. phosphatase signal sequence in)

INDEX TERM: Interferons
 (.alpha.-, gene for, of human, cloning in **Escherichia coli** of)

INDEX TERM: Gene and Genetic element, microbial
 (**phaA**, promoter of, of **Escherichia coli**, in human **.alpha.-interferon** gene expression)

INDEX TERM: 9001-78-9
 (promoter and signal sequence for, of **Escherichia coli**, in human **.alpha.-interferon** gene expression)

Exmr: C. Smith (AU 1812)

=> d clster .bio;d que 133;d rank;file hits
 DISPLAY L# IS NOT VALID IN STNINDEX

COMMAND STACK INTERRUPTED. ENTER "DISPLAY HISTORY"
 TO SEE WHICH COMMANDS WERE EXECUTED.

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=> d cluster .bio;d que 133;d rank;file hits
CLUSTER NAME      CLUSTER DEFINITION
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.BIO               MEDLINE HCA EMBASE BIOSIS WPIDS IFIPAT BIOTECHDS
                  DISSABS CONFSCI LIFESCI SCISEARCH JAPIO
                  JICST-EPLUS

L28                QUE   (142192-09-4 OR ALPHA(1A) INTERFERON)
L29                QUE   L28 AND (ESCHERICHIA COLI OR E COLI)
L30                QUE   (HEAT(2A) STABLE(2A) TOXIN# OR STII OR ST11 OR STABL
                  E(2A) ENTEROTOXIN#)
L31                QUE   L29 AND L30
L32                QUE   (PHOA OR ALKALINE(2A) PHOSPHATASE(2A) PROMOTER) AND
                  L29
L33                QUE   L31 OR L32

F1                 2     MEDLINE
F2                 2     EMBASE
F3                 2     BIOTECHDS
F4                 1     BIOSIS
F5                 1     WPIDS
F6                 1     LIFESCI
F7                 1     SCISEARCH
```

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=> d que 143
 L28 QUE (142192-09-4 OR ALPHA(1A) INTERFERON)

SN:08/249,671(Srch. by Dilip 308-4268)

L29 QUE L28 AND (ESCHERICHIA COLI OR E COLI)
L30 QUE (HEAT(2A) STABLE(2A) TOXIN# OR STII OR ST11 OR STABL
E (2A) ENTEROTOXIN#)
L31 QUE L29 AND L30
L32 QUE (PHOA OR ALKALINE(2A) PHOSPHATASE(2A) PROMOTER) AND
L29
L33 QUE L31 OR L32
L34 2 SEA FILE=MEDLINE L31 OR L32
L35 2 SEA FILE=EMBASE L31 OR L32
L36 2 SEA FILE=BIOTECHDS L31 OR L32
L37 1 SEA FILE=BIOSIS L31 OR L32
L38 1 SEA FILE=WPIDS L31 OR L32
L39 1 SEA FILE=LIFESCI L31 OR L32
L40 1 SEA FILE=SCISEARCH L31 OR L32
L41 10 SEA L33
L42 3 DUP REM L41 (7 DUPLICATES REMOVED)
L43 3 SOR L42 PY

=> d bib ab 1-

L43 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 1995 DERWENT INFORMATION LTD
 AN 95-01432 BIOTECHDS
 TI Interferon-alpha production in
Escherichia coli with periplasmic secretion;
 protein secretion and purification; DNA sequence and protein
 sequence
 AU Hauptmann R; Falkner E; Bodo G; Voss T; Maurer-Fogy I
 PA Boehr Ingelheim
 PI EP-626448 30 Nov 1994
 AI 94EP-0107804 19 May 1994
 PRAI 93DE-4329756 3 Sep 1993; 93DE-431745P 26 May 1993
 DT Patent
 LA German
 OS WPI: 95-000932 [01]
 AB Production of interferon-alpha (I) in
Escherichia coli involves growing cells that
 contain a vector in which the signal peptide (A) of the gene for
E. coli thermostable enterotoxin-II (**STII**)
) is coupled to a sequence (B) encoding human mature (I). The
 following are also claimed: purification of (I) by adsorption
 chromatography on silica gel, hydrophobic interaction
 chromatography, cation-exchange chromatography and anion-exchange
 chromatography; and vectors for expressing (I) where (A) is linked
 to (B). Attachment of (A) to (B) ensures a stable expression
 system that ensures correctly folded protein secretion into the
 periplasmic space. Preferably, the vector includes a promoter from
 the **E. coli** alkaline phosphatase gene and a
 ribosome binding site from the **STII** gene. (B) preferably
 encodes interferon-alpha-2c of specified
 protein sequence. The 879 bp DNA sequence encoding this peptide
 preceded by the **STII** signal peptide is specified. When
 the (A)-(B) construct is used under the control of the
alkaline phosphatase promoter,
 expression can be controlled by altering the phosphate level in the
 culture medium. (28pp)

L43 ANSWER 2 OF 3 MEDLINE
 AN 85289134 MEDLINE
 TI Secretion of human interferon-alpha induced by
 using secretion vectors containing a promoter and signal sequence of
 alkaline phosphatase gene of **Escherichia coli**.
 AU Miyake T; Oka T; Nishizawa T; Misoka F; Fuwa T; Yoda K; Yamasaki M;
 Tamura G
 SO J Biochem (Tokyo), (1985 May) 97 (5) 1429-36.
 Journal code: HIF. ISSN: 0021-924X.
 CY Japan
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 8512
 AB We constructed a new vector containing the promoter and the signal
 sequence of **E. coli phoA** gene, the
 structural gene for the periplasmic alkaline phosphatase. One of the
 most useful characteristics of this vector is the unique HindIII

restriction site located just at the end of the **phoA** signal sequence. This restriction site was generated by oligonucleotide-directed site-specific mutagenesis without changing the amino acid sequence of the signal peptide. Any kind of foreign structural gene can be easily inserted into the HindIII site by using synthetic oligonucleotides to construct a hybrid gene which has neither an extra sequence nor a deletion between the **phoA** signal sequence and the foreign structural gene. Human **alpha-interferon** gene was inserted into this HindIII site. When this hybrid gene was expressed under the control of the **phoA** promoter region, a low but significant activity was recovered in the cold water wash of the cells after an osmotic shock procedure.

L43 ANSWER 3 OF 3 MEDLINE

AN 94190282 MEDLINE

TI Periplasmic expression of human **interferon-alpha** 2c in **Escherichia coli** results in a correctly folded molecule.

AU Voss T; Falkner E; Ahorn H; Krystek E; Maurer-Fogy I; Bodo G; Hauptmann R

CS Ernst-Boehringer Institut fur Arzneimittelforschung, Bender & Co., Vienna, Austria.

SO Biochem J, (1994 Mar 15) 298 Pt 3 719-25.
Journal code: 9YO. ISSN: 0264-6021.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9406

AB Human **interferon-alpha** 2c (IFN-alpha 2c) was produced in **Escherichia coli** under the control of the **alkaline phosphatase promoter** using a periplasmic expression system. Compared with other leader sequences, the **heat-stable enterotoxin II** leader of **E. coli** (**STII**) resulted in the highest rate of correct processing as judged by Western-blot analysis. The fermentation was designed as a batch-fed process in order to obtain a high yield of biomass. The processing rate of IFN-alpha 2c could be increased from 25% to more than 50% by shifting the fermentation pH from 7.0 to 6.7. IFN-alpha 2c extracted from the periplasm was purified by a new four-step chromatographic procedure. Whereas cytoplasmically produced IFN-alpha 2c does not have its full native structure, IFN-alpha 2c extracted from the periplasm was found to be correctly folded, as shown by c.d. spectroscopy. Peptide-map analysis in combination with m.s. revealed the correct formation of disulphide bridges. N-terminal sequence analysis showed complete removal of the leader sequence, creating the authentic N-terminus starting with cysteine.

=> file hom;d his

FILE 'HOME' ENTERED AT 15:18:38 ON 30 AUG 95

(FILE 'HOME' ENTERED AT 14:40:23 ON 30 AUG 95)
SET PAGELENGTH SCROLL

FILE 'REGISTRY' ENTERED AT 14:41:00 ON 30 AUG 95

E INTERFERON ALPHA/CN
E INTERFERON ALPHA/CN
E ALPHA INTERFERON/CN

FILE 'HCA' ENTERED AT 14:41:58 ON 30 AUG 95

L1 2639 S (ALPHA(1A)INTERFERON)/IT
E HAUPTMANN RUDOLF/AU
L2 29 S HAUPTMANN RUDOLF/AU
L3 6 S L1 AND L2
L4 29763 S INTERFERON#/IA, IT, ST

FILE 'HOME' ENTERED AT 14:46:41 ON 30 AUG 95

FILE 'HCA' ENTERED AT 14:49:01 ON 30 AUG 95

L5 33396 S PLASMID AND EPISOME/IT
L6 61528 S L5 OR (PLASMID# OR EPISOME#)/IA, IT, ST
L7 984 S L4 AND L6
L8 3 S L7 AND (142192-09-4 OR 142192-09-4D OR 142192-09-4P)
L9 2 S L8 NOT L3

FILE 'HCA' ENTERED AT 14:51:45 ON 30 AUG 95

L10 131526 S (ESCHERICHIA COLI OR E COLI)/IA, IT, ST
L11 1366 S L4 AND L10
L12 53776 S (TOXIN#)/IA, IT, ST
L13 87 S L11 AND L12
L14 524215 S (HEAT(2A)STABLE(2A)TOXIN# OR ST## OR STABLE(2A)ENTEROTO
L15 7 S L13 AND L14
L16 6 S L15 NOT L3
L17 884 S (HEAT(2A)STABLE(2A)TOXIN# OR STII OR ST11 OR STABLE(2A)
L18 3 S L13 AND L17
L19 2 S L18 NOT L3
L20 2 SORT L19 PY

FILE 'HCA' ENTERED AT 14:58:20 ON 30 AUG 95

L21 4930 S (ALPHA(1A)INTERFERON)/IA, IT, ST
L22 4930 S (142192-09-4 OR 142192-09-4D OR 142192-09-4P)/IA, IT, ST
L23 360 S L22 AND L10
L24 354 S L23 NOT L3
L25 0 S L24 AND L17
L26 496 S (PHOA OR ALKALINE(2A)PHOSPHATASE(2A)PROMOTER#)/IA, IT, ST
L27 1 S L24 AND L26

FILE 'HCA' ENTERED AT 15:03:54 ON 30 AUG 95

INDEX 'MEDLINE, HCA, EMBASE, BIOSIS, WPIDS, IFIPAT, BIOTECHDS,
DISSABS, CONFSCI, LIFESCI, SCISEARCH, JAPIO, JICST-EPLUS' ENTERED
AT 15:05:00 ON 30 AUG 95

INDEX 'MEDLINE, EMBASE, BIOSIS, WPIDS, IFIPAT, BIOTECHDS, DISSABS, CONFSCI, LIFESCI, SCISEARCH, JAPIO, JICST-EPLUS' ENTERED AT
15:05:06 ON 30 AUG 95
SEA (ALPHA(1A) INTERFERON)

8199 FILE MEDLINE
SEA (142192-09-4 OR ALPHA(1A) INTERFERON)

8199 FILE MEDLINE
12810 FILE EMBASE
11773 FILE BIOSIS
320 FILE WPIDS
143 FILE IFIPAT
702 FILE BIOTECHDS
131 FILE DISSABS
429 FILE CONFSCI
3011 FILE LIFESCI
7671 FILE SCISEARCH
59 FILE JAPIO
2304 FILE JICST-EPLUS

L28 QUE (142192-09-4 OR ALPHA(1A) INTERFERON)

SEA L28 AND (ESCHERICHIA COLI OR E COLI)

182 FILE MEDLINE
183 FILE EMBASE
239 FILE BIOSIS
36 FILE WPIDS
13 FILE IFIPAT
284 FILE BIOTECHDS
3 FILE DISSABS
5 FILE CONFSCI
95 FILE LIFESCI
105 FILE SCISEARCH
3 FILE JAPIO
12 FILE JICST-EPLUS

L29 QUE L28 AND (ESCHERICHIA COLI OR E COLI)

SEA (HEAT(2A) STABLE(2A) TOXIN# OR STII OR ST11 OR STABLE(2

1046 FILE MEDLINE
829 FILE EMBASE
1240 FILE BIOSIS
33 FILE WPIDS
15 FILE IFIPAT
71 FILE BIOTECHDS
48 FILE DISSABS
49 FILE CONFSCI
507 FILE LIFESCI
757 FILE SCISEARCH
49 FILE JAPIO
57 FILE JICST-EPLUS

L30 QUE (HEAT(2A) STABLE(2A) TOXIN# OR STII OR ST11 OR STABLE

SEA L29 AND L30

```

1 FILE MEDLINE
1 FILE EMBASE
1 FILE BIOSIS
1 FILE WPIDS
1 FILE BIOTECHDS
1 FILE SCISEARCH
L31    QUE L29 AND L30
-----
SEA (PHOA OR ALKALINE(2A) PHOSPHATASE(2A) PROMOTER) AND L29
-----
2 FILE MEDLINE
2 FILE EMBASE
1 FILE BIOSIS
1 FILE WPIDS
2 FILE BIOTECHDS
1 FILE LIFESCI
1 FILE SCISEARCH
L32    QUE (PHOA OR ALKALINE(2A) PHOSPHATASE(2A) PROMOTER) AND L
-----
SEA L31 OR L32
-----
2 FILE MEDLINE
2 FILE EMBASE
1 FILE BIOSIS
1 FILE WPIDS
2 FILE BIOTECHDS
1 FILE LIFESCI
1 FILE SCISEARCH
L33    QUE L31 OR L32
-----
FILE 'MEDLINE, EMBASE, BIOTECHDS, BIOSIS, WPIDS, LIFESCI,
SCISEARCH' ENTERED AT 15:15:03 ON 30 AUG 95
FILE 'MEDLINE'
L34      2 S L33
FILE 'EMBASE'
L35      2 S L33
FILE 'BIOTECHDS'
L36      2 S L33
FILE 'BIOSIS'
L37      1 S L33
FILE 'WPIDS'
L38      1 S L33
FILE 'LIFESCI'
L39      1 S L33
FILE 'SCISEARCH'
L40      1 S L33
TOTAL FOR ALL FILES
L41      10 S L33
L42      3 DUP REM L41 (7 DUPLICATES REMOVED)
L43      3 SORT L42 PY

```

FILE 'HOME' ENTERED AT 15:18:38 ON 30 AUG 95